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An Animal Model of Drug-Induced Thermoregulatory and Endurance Decrements

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INTRODUCTION

Effects of the neurotransmitter acetylcholine are divided into muscarinic or nicotinic depending on the receptors that are activated. Stimulation of muscarinic receptors results in vasodilation, decreased heart rate and cardiac contractility, increased secretions of exocrine glands including sweat and salivary, increased intestinal and gastric contractions, and increased bronchial secretions. Agonists at nicotinic receptors stimulate autonomic ganglia and neuromuscular junctions. Over-stimulation results in asynchronous excitation, fatigability, and involuntary twitching[1]. Anticholinergic drugs are commonly used as antihistamines, tranquilizers, cold medications, antidiarrheal medications, as well as treatments for motion sickness and anticholinesterase poisoning. Use of anticholinergic drugs has detrimental effects on thermoregulation particularly in hot environments due to sweat suppression[2]. Anticholinesterase drugs are used clinically for treatment of myasthenia gravis, Alzheimer's disease, anticholinergic syndrome, and as a pretreatment against potential organophosphorus exposure[1].

Earlier work from this laboratory established the exercising rat and the sedentary heat-stressed rat as models of human heat-induced injury[3,4,5,6]. More recently, interest centered on the effects of anticholinergic and anticholinesterase drugs on physical, physiological, and thermoregulatory responses to hot environments and exercise in moderate to hot environments. Atropine, the prototypical anticholinergic drug, inhibits evaporative cooling by suppressing sweating in man[1,7,8] and by suppressing saliva secretion in rats which is behaviorally spread for evaporative cooling[9,10]. Clubley *et al.*[11] reported that sweat and saliva production are both analogously regulated in man. A 2 mg dose of atropine in a 70 kg man has been shown to suppress the sweat rate by about

40%[12,13,14,15]. Our work demonstrated that a 200 ug/kg dose of atropine (equivalent to 2 mg in man[16]) inhibits salivary water loss by the same 40%.

Sweat in man[17] and saliva in rats[18] contain Na^+ and K^+ but are hypotonic with respect to serum. Thus, both man[19] and rats[20] lose electrolytes, are dehydrated, and have increased serum osmolarities as a result of prolonged evaporative cooling. It has long been known that thirst is an inadequate stimulus for rehydration in man[21]. Recently, Barney and West[20] reported that thermally-induced salivary loss in rats increases plasma osmolality and that thirst is stimulated only to the point of replacing 50% of the water when the osmolality returns to control levels. Thus, mechanisms of evaporative cooling in man and rats are similar in cooling fluid composition, neural control, and resultant effects on dehydration and thirst.

MODEL DESCRIPTION

Experimental Animals: Adult male Sprague-Dawley (Charles River CD strain) 510-530 g rats were used for all of the studies unless otherwise indicated. The animals were caged individually and housed in an environmental chamber maintained at 26°C and 50% rh. Lighting was controlled automatically (on, 0600-1800 hours) and rat chow and water were available ad lib. except during experimental intervals. Drug administration and blood sampling, when required, were accomplished using a lateral tail vein or a jugular cannula surgically implanted in rats under sodium pentobarbital anesthesia.

Sedentary Heat-Stressed Rats: Except as noted below, rats were unrestrained during heat stress to facilitate saliva spreading activity. Rats, in their own cages, were placed in an environmental chamber maintained at 41.5°C until the desired end point core temperature (Tc) was attained (usually 42.6°C), when the animals were removed to a 26°C chamber. During heat stress, Tc, weight loss (as a measure of fluid spread for evaporative cooling), and the extent of saliva or urine spread were monitored. Fecal pellet production was monitored both to correct weight loss to more accurately reflect water loss and as an indication of intestinal motility.

When saliva is unavailable as in the case of surgical desalivation[10] or inhibition of salivation by anticholinergic drugs[10,22,23], rats will spread urine if no other fluid is available. We reported[22] (Fig. 1) that the extent of saliva or urine spread for evaporative cooling was a useful measure of the surface-available for

evaporative cooling. Since, saliva-spreading requires both physiological and grooming activity, this provides a behavioral as well as physiological measure.

Exercising Rats: To identify and quantify effects on exercise endurance and thermoregulation, rats were run on a motor driven treadmill (11 m/min, 6° incline, shock avoidance contingency). Unless otherwise indicated, they were run to exhaustion, defined as the point at which rats fail to keep pace on the treadmill and do not immediately right themselves when placed on their backs. Rats were run for 20 min then allowed a 2 min rest, run for an additional 20 min followed by a 2 min rest and then run to exhaustion without any further rest (control animals ran for about 60 min). During running and subsequent recovery Tc (thermistor probe inserted 6.5 cm beyond the anal sphincter) as well as tail temperature (Tt, surface thermistor taped to the dorsal mid point of the tail) were monitored with a computerized data acquisition system.

For studies of anticholinesterase drug effects, a drug symptom check list[24] was developed to quantitate tremors, salivation, exophthalmus, defecation, run performance, and overall behavior. These indices proved useful in identifying the efficacy, dosages, and specificity of anticholinergic drugs[24,25].

RESULTS

Sedentary Heat-Stressed Rats: Following are descriptions of results reported using the sedentary heat-stressed rat model in our laboratory[10,22,23,30]:

Prior to using the heat-stressed rat as a model for examining the thermoregulatory effects of anticholinergic drugs, it was necessary to compare the response of heat-stressed rats that were unable to spread saliva for evaporative cooling with the response of heat-stressed control animals. In this study,[10] rats were surgically desalivated (DESAL) by ligation and transection of the salivary ducts. Since surgically desalivated rats were found to spread urine for evaporative cooling when no other fluid was available, it was necessary to restrain (RES) these animals to prevent spread of any fluid for evaporative cooling. Unrestrained (UNRES) control animals (Table 1) spread saliva on their ventral surface losing 8% of their body weight (Tc final= 42.6°C) with a rate of rise of core temperature (heating rate) of 0.02°C/min. Restraint (CONTROL RES) inhibited, but did not completely prevent, behavioral spread of saliva; some saliva was groomed into the fur and some dripped out of the mouth and was lost. DESAL UNRES rats spread some urine resulting in similar % weight loss and heating rate to those of the

CONTROL RES rats. A combination of desalivation and restraint was required to achieve heat stress with no evaporative cooling; DESAL RES rats had the minimum % weight loss and maximum heating rate. Pharmacological desalivation via administration of 1 mg/kg of atropine prior to the start of heat stress resulted in inhibition of both salivation and urine spreading with % weight loss and heating rate similar to those of the DESAL RES rats. Thus, Hubbard, *et al.*[10] concluded that the heat-stressed rat was a potentially useful model of anticholinergic-induced thermoregulatory decrements.

TABLE 1

Weight Loss and Heating Rate of Surgically and Chemically Desalivated Rats

Group [a]	% Weight Loss	Heating Rate, °C/min
CONTROL UNRES	8.0 ± 0.4	0.02 ± 0.002
CONTROL RES	2.2 ± 0.2	0.08 ± 0.01
DESAL UNRES	1.9 ± 0.4	0.07 ± 0.01
DESAL RES	0.7 ± 0.1	0.16 ± 0.01
ATROPINE 1mg/kg	0.7 ± 0.1	0.12 ± 0.004

a Data from Hubbard, *et al.*[10]; see text for group definitions.

In a subsequent study[22], the dose-response and pharmacokinetic effects of atropine were examined in the sedentary heat-stressed rat. Doses of atropine ranging from 10-4000 ug/kg were administered via tail vein 15 min prior to the start of heat stress. In the range of 25 to 1000 ug/kg atropine was found to increase heating rate and decrease % weight loss and fecal pellet production (anticholinergics decrease intestinal motility) in a dose-response manner. A 200 ug/kg dose (equivalent to a 2 mg dose in man[16]) resulted in a 38% decrease in water loss rate which was similar to the 42%[12] and 43%[15] seen in man. The pattern of saliva or urine spread was also found to have a dose-response relationship to atropine administration. Despite some individual variability, the patterns in Fig 1 were found to be consistent with the following: S-1, 200 ug/kg atropine; S-2, 50 ug/kg; S-3, 10 ug/kg or saline controls; U-1, 500 ug/kg; U-2 or U-3, surgically desalivated saline controls (from the previous study[10]). In this same

study, 250g rats were found to be less sensitive to dose-response effects of atropine administration than 500 g rats. Thus, the larger rats were used in all studies. This study also examined the effect of increasing the time interval between atropine administration and the start of heat stress. While intestinal motility returned to normal levels after a 60 min delay, and % weight loss returned to control levels after a 120 min delay, heating rate required a 210 min interval to return to control levels. These times correspond closely with the 2-4 hour time required for atropine effects to subside in man[26,27,28,29]. Based on the similarity of the rat and human responses to atropine, it was determined that the sedentary heat-stressed rat was a promising model with which to examine relative anticholinergic drug effects.

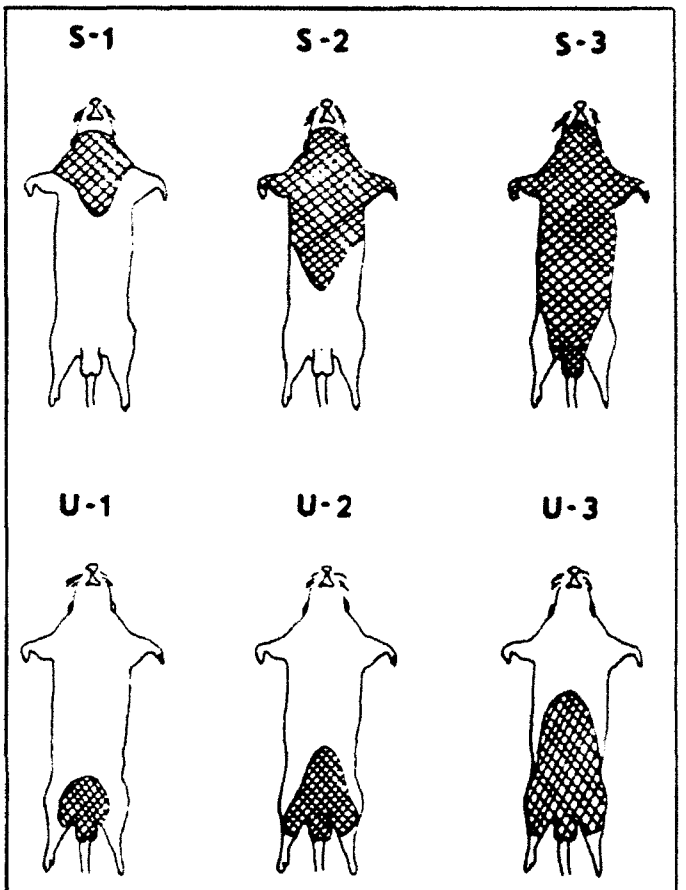

Fig. 1 Typical progressive saliva (S-1, S-2, and S-3) spread pattern of control rats and urine (U-1, U-2, and U-3) spread of atropinized or surgical desalivated rats. (Matthew *et al.*[21])

Since atropine is frequently administered intramuscularly (im) rather than intravenously (iv), a comparison of iv and im routes of administration in the heat-stressed rat was undertaken[30]. The effects of im or iv atropine were quantified on the following variables: heating rate, % weight loss, fecal loss (intestinal motility), and mydriasis (in rats that were not heat stressed.) Both iv and im atropine had similar effects on weight loss (decreasing with increasing atropine dose) and mydriasis (pupil dilation to a greater extent and longer duration with increasing dose.) The range of doses of atropine (10-1000 ug/kg) over which there was a dose-response increase in heating rate with iv administration was truncated with im administration (10-50 ug/kg). Therefore, we concluded that the iv route was the better route for examining anticholinergic-induced inhibition of evaporative cooling.

TABLE 2

ANTICHOLINERGIC POTENCY RELATIVE TO ATROPINE

Drug	Dose (ug/kg)	Heating Rate (°C/min)[a]	Potency Ratio[b]	AT=Dose (ug/kg)[c]
Saline[d]	----	0.022 ± 0.001	----	----
Atropine[d]	200	0.087 ± 0.004	1	200
Atropine Methyl Nitrate[d]	25	0.067 ± 0.006	4	50
Scopolamine[d]	10	0.079 ± 0.005	16	12
Chlorpromazine[d]	1000	0.067 ± 0.004	0.1	2000
L-Hyoscyamine[d]	100	0.089 ± 0.004	2	100
Aprophen[e]	2000	0.072 ± 0.004	0.067	3000
Trihexyphenidyl[e]	400	0.033 ± 0.004	0.061	3200

a Values are mean ± standard error.
b Calculated potency relative to atropine from Matthew, *et al.*[23]
c 200 ug/kg AT/potency ratio; drug dose required to elicit the same heating rate in the heat-stressed rat as 200 ug/kg of AT.
d Values taken from Matthew *et al.*[23]
e Values taken from Matthew[24].

Since the heating rate of sedentary heat-stressed rats was the most sensitive index of anticholinergic activity, the heating rates of animals given other anticholinergic drugs were compared to the dose-response increase in heating rate induced by atropine administration[23]. An atropine equivalent dose and an anticholinergic potency relative to a value of 1 for atropine were determined as indicated in Table 2. Additionally, the relative abilities of carbamates to reverse the atropine-induced increase in heating rate as a measure of relative anticholinesterase potency were compared. Neostigmine was determined to have a value of 8 relative to 2 for physostigmine and 1 for pyridostigmine[23]. Thus, the core temperature response of sedentary heat-stressed rats can be used as a sensitive bioassay for both anticholinergic and anticholinesterase efficacy.

Exercising Rats: In the sedentary heat-stressed rat, the cholinergic drug effects examined were primarily muscarinic (salivation, intestinal motility, vasodilation, etc.). However, the exercising rat is unable to spread secreted saliva while exercising; thus, nicotinic cholinergic effects (primarily at the neuromuscular junction) can be evaluated. Following are descriptions of cholinergic drug effects using an exercising rat model[24,25,32,33,34,38,39,40,41].

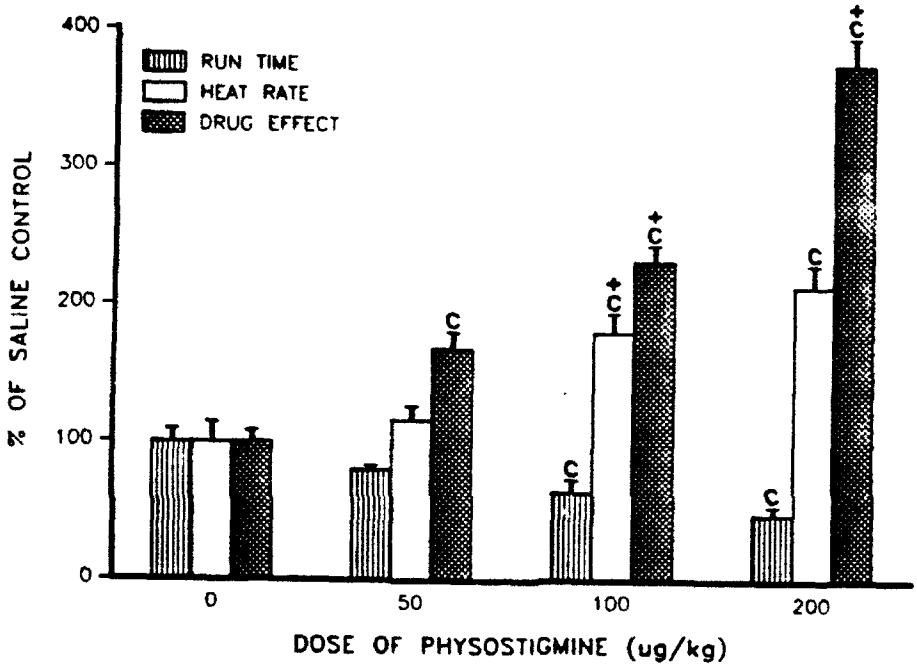

Fig. 2 Run time, heating rate, and cholinergic drug effect score for rats receiving saline (0), 50, 100, 200 ug/kg of PH are plotted as a % of saline control values. Significant difference from control value = "C", from previous lower dose = "+". (From Matthew, *et al.*[25]).

Physostigmine, (PH) a reversibly acting carbamate anticholinesterase, was initially chosen to establish dose-response drug effects in the running rat. PH was selected because it is a drug in clinical use, has central as well as peripheral sites of action[1], and has been shown to be protective against organophosphorus poisoning[31]. In the results illustrated in Fig. 2[25], PH was administered via tail vein 15 min prior to the start of run. Control animals had a run time of 67 $\pm$ 6 min (mean $\pm$ S.E.), a heating rate of 0.051 $\pm$ 0.007°C/min, and cholinergic symptom

score of 2.5 $\pm$ 0.2. Endurance declined as heating rate and cholinergic symptoms increased with increasing doses of PH. Since endurance, thermoregulatory effects, and cholinergic symptoms all vary in a dose-dependent manner with PH, this exercising rats model represents a potentially useful model with which to examine other anticholinesterases or anticholinergics.

In order to alleviate PH-induced thermoregulatory and endurance decrements in running rats, a combination of atropine (muscarinic anticholinergic) and diazepam (anticonvulsant) was administered with PH to running rats[32]. The administration of atropine (200 ug/kg) and diazepam (500 ug/kg) with PH (200 ug/kg) resulted in run times and heating rates which were not significantly different from those of saline controls. In a separate study[33], subchronic (2 week) administration of PH (60% inhibition of cholinesterase (ChE)) resulted in endurances and heating rates which were not significantly different from controls. In the same study[33] subchronic PH administration resulted in attenuation of ultrastructural changes commonly seen with acute PH administration. Accommodation to excess acetylcholine at receptors was noticeable after one week of PH administration via osmotic pump and complete after 2 weeks.

Subsequently, the sedentary heat-stressed rat model and the exercising rat model were used to determine optimal doses of 4 anticholinergic drugs to neutralize the PH-induced decrements in exercising rats[24]. The optimum dose of atropine (200 ug/kg) was determined by administering a range of doses of AT with PH prior to exercise. Doses of AT either higher or lower than 200 ug/kg elicited higher heating rates and cholinergic symptom scores and lower endurance times. Doses of scopolamine (S), aprophen (AP), and trihexyphenidyl (THP) equivalent to 200 ug/kg of AT **in the sedentary heat-stressed rat** were determined by the method[23] indicated above (Table 2) to be S- 12, AP- 3000, and THP- 3200 ug/kg. In the **exercising rat** model, the optimum dose of S (12 ug/kg) and AP (3000 ug/kg) as adjuncts to PH were determined to be the same doses that were equivalent **in the sedentary heat-stressed rat** to the dose of AT (200 ug/kg) found to be optimum in the **exercising rat.** AT, S, and AP are primarily muscarinic anticholinergic drugs. **The optimal dose of THP (800 ug/kg) in the exercising rat** is only 1/4 of the dose equivalent to the standard AT dosage in the **sedentary heat-stressed rat. This could be explained because THP selectively blocks only** M-1 (central, neuronal) cholinergic receptors, while AT, S, and AP all block both M-1 and M-2 (secretory, smooth muscle, and cardiac) receptors[1,34,35].

The peripheral vasodilatory effect[36,37] of PH and other centrally acting anticholinesterases may result in decreased core temperature[25,32,36,37]. In sedentary

animals this peripheral vasodilation results in a decrease in core temperature at or below an ambient temperature of 25°C[25,32,37] which should confer a thermoregulatory advantage. However, when PH-treated animals are exercised at 26°C, they have an increased heating rate[24,25,32,33]. The cause of this increased heating was postulated to be increased acetylcholine and metabolic activity at the neuromuscular junction. To elucidate the mechanism involved in PH-induced thermoregulatory decrements, rats were exercised after treatment with PH at ambient temperatures of 10, 15, 26, and 30°C[38]. Results indicated that 10°C may be low enough to allow sufficient radiant cooling in both saline and PH-treated groups, that 30°C is too high for effective cooling in either group, and that at 15 and 26°C the PH-treated animals had significantly increased rates of rise of core temperature. Thus, the PH-treated rat model is useful over a temperature range of 15-26°C.

The quaternary carbamate pyridostigmine (PY) was fielded and used by the military in Desert Storm as a nerve agent pretreatment drug. At low to moderate clinical doses PY does not cross the blood-brain barrier in pharmacologically significant amounts[1]; therefore, its effects can be attributed to peripheral sites of action. PY acutely administered to exercising rats (Tamb= 35°C, 40-60% ChE inhibition) decreased endurance and increased heating rates[39]. Chronic oral PY administration (20-40% ChE inhibition), also in rats exercising in a warm environment, did not decrement endurance or thermoregulation[40] indicating that, as was the case with PH, there may be accommodation with chronicity. Additionally, the endurance and thermoregulatory decrements induced by acute PY administration (40% ChE inhibition, 26°C) were attenuated by AT and diazepam administration[41].

CURRENT AND POTENTIAL APPLICATIONS

If PY is given prophylactically, it will be given subchronically which will result in consistently elevated acetylcholine levels at receptors. This chronic elevation may result in accommodation and eliminate adverse effects seen with acute administration as was the case with PH[33]. However, if there is an alteration in receptor sensitivity following subchronic PY, acute administration of atropine subsequent to toxic exposure may not result in anticipated levels of anticholinergic activity[23,42]. An evaluation of the effects of subchronic PY with acute AT administration is currently underway in our laboratory.

The acute administration of either PH or PY resulted in endurance and thermoregulatory decrements in exercising rats[32,39,41]. This is hypothesized to be due to increased metabolic activity at the neuromuscular junction[24] for the following reasons: 1) The decrements are peripheral in origin because both the PH and PY elicit the same decrements. 2) Preliminary results from this laboratory indicate that PH administration to sedentary rats results in an increased oxygen consumption despite the drop in core temperature subsequent to tail vasodilation. 3) PH- or PY-stimulated salivary secretion does not contribute to cooling while rats are running on the treadmill. Since none of the anticholinergic drugs used to block PH-induced decrements is a specific nicotinic blocker[24], conclusive evidence for this hypothesis awaits use of ganglionic and neuromuscular junction nicotinic blockers with PH in the exercising rat.

Because of the similarities between sweat loss dehydration in man and salivary loss dehydration in the rat, the sedentary heat-stressed rat model is currently being used to examine treatment of hyperthermic dehydration with hypertonic saline in dextran (HSD). This new use of the model increases its potential usefulness for examining problems in military medicine where adequate hydration is a chronic problem. With the existing data base on the exercising and sedentary heat-stressed rat models and current work to identify specific sites of anticholinesterase-induced decrements, these models are available for evaluating prospective new therapies as they are identified.

The views, opinions, and/or findings contained in this report are those of the author and should not be construed as official Department of the Army position, policy or decision, unless so designated by other official documentation. In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as prepared by the committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council. Citations of commercial organizations and trade names do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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